

50-65 from E1 protein HPV16 and additionally, amino acid residues 384-403 from E1 protein of HPV16 were fused at the N-terminus corresponding to sequence (B).

For generation of recombinant (A), the sequence of HPV16 L1 ORF was amplified by polymerase chain reaction (PCR) from a total DNA extract of WI 2, an HPV16 episome-containing cell line (Stanley M, Brown HM, Appleby M, and Minson AC (1989) "Properties of a non-tumorigenic human keratinocyte cell line", Int J Cancer 43, 672-676) using a forward primer in which a Bgl I 11 restriction sequence was included (underlined) 5'-GCT GCA AGA TCT ATG TCT CTT TGG CTG CCT AG-3' (SEQ ID NO: 1).

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For generation of recombinant (B), a DNA nucleotide sequence encoding the E1 50-65 sequence plus a Bgl I 11 restriction sequence (underlined) was introduced just in front of the L1 coding sequence as a forward primer, as follows: 5'-GCT GCA AGA TCT ATG GTA GAT TTT ATA GTA AAT GAT AAT GAT TAT TTA ACA CAG GCA GAA TCT CTT TGG CTG CCT AGT GAG-3' (SEQ ID NO: 2).

For generation of recombinant (C), a nucleotide sequence-encoding E1 amino acids 384-403 was introduced just in front of E1 50-65 coding sequence of the last-mentioned construct, using a forward primer with a flanking Bgl I 11 restrictional sequence (underlined): 5'-GCT GCA AGA TCT ATG TAC GAT AAT GAC ATA GTA GAC GAT AGT GAA ATT GCA TAT AAA TAT GCA CAA TTG GCA GAC GTA GAT TTT ATA GTA AAT GAT-3' (SEQ ID NO: 3).

These forward primers were paired with the same reverse primer in which a Not I restriction site was included (underline). Reverse: 5'-GAT CTA GCG GCC GC TTA CAG CTT ACG CTT CTT GCG TTT-3' (SEQ ID NO: 4).

Following 30 cycles of amplification, the DNA products (e.g. of about 1.7kb in size) were gel purified, GENECLAN (TM) excised, digested with restriction enzymes of Bgl I 11 and Not I and sub-cloned into baculovirus transfer vector pBacAK8 (Clontech) which had